Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXIV, No. 11

SYNTHESIS OF CARBON-14 LABELLED SPIRO-PIPERIDYL-RIFAMYCINS (LM 118) AND RIFABUTIN (LM 427)

Erminia Fontana, Silvio Giarda*, Sergio Vioglio and Gian Piero Vicario

Farmitalia Carlo Erba S.p.a.
Chemical Research and Development
Via Giovanni XXIII, 23 - 20014 Nerviano, Milano, Italy

SUMMARY

Two syntheses of radiolabelled spiro-piperidyl-rifamycins are described.

1. A five steps synthesis (Scheme 1) was performed to give 4-deoxo-3,4-[2-spiro-(N-[^{14}C]methyl-4-piperidyl)]-(1H)-imidazo-(2,5-dihydro)-rifamycin S ([^{14}C]LM 118), in an overall radiochemical yield of ~20%, 98% radiochemically pure and with a specific activity of 440 MBq/mmol (11.9 mCi/mmol) starting from [^{14}C]methyl iodide 1.
2. A three steps synthesis (Scheme 2) was performed to give 4-deoxo-3,4-[2-spiro-(N-2-methyl[1-^{14}C]propane-4-piperidyl)]-(1H)-imidazo-(2,5-dihydro)-rifamycin S ([^{14}C] rifabutin) in an overall radiochemical yield of ~38%, 97% radiochemically pure with a specific activity of 1.27 GBq/mmol (34.32 mCi/mmol). 1-(2-Methyl [1-^{14}C]propanoyl)-piperazin-4-one ethylene ketal was employed as starting material.

Key Words: [^{14}C]-spiro-piperidyl-rifamycin S, LM 118, rifabutin, LM 427, antibacterial.

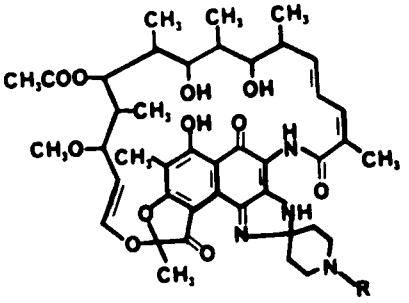
* Current address: Istituto G. Donegani - Centro Ricerche - Novara - Italy.

0362-4803/87/111341–09$05.00

Received January 19, 1987
Revised March 16, 1987

INTRODUCTION

The spiro-piperidyl-rifamycins are a new class (1) of rifamycin S antibiotics endowed with antibacterial activity (2,3). In a recent paper Marsili et al. (1) described many derivatives of rifamycin and among these the spiro-piperidyl compounds LM 118 and rifabutin (LM 427) showed a promising activity against atypical Mycobacterium tubercolosis strains (2,3).



R = CH3 8, (LM 118)
R = CH_2-CH-$(CH3)_2$ 12, (rifabutin)

The requirement of radioactive derivatives to assist disposition, metabolism and pharmacokinetics studies in laboratory animals, prompted us to prepare these substances in a suitable labelled form.

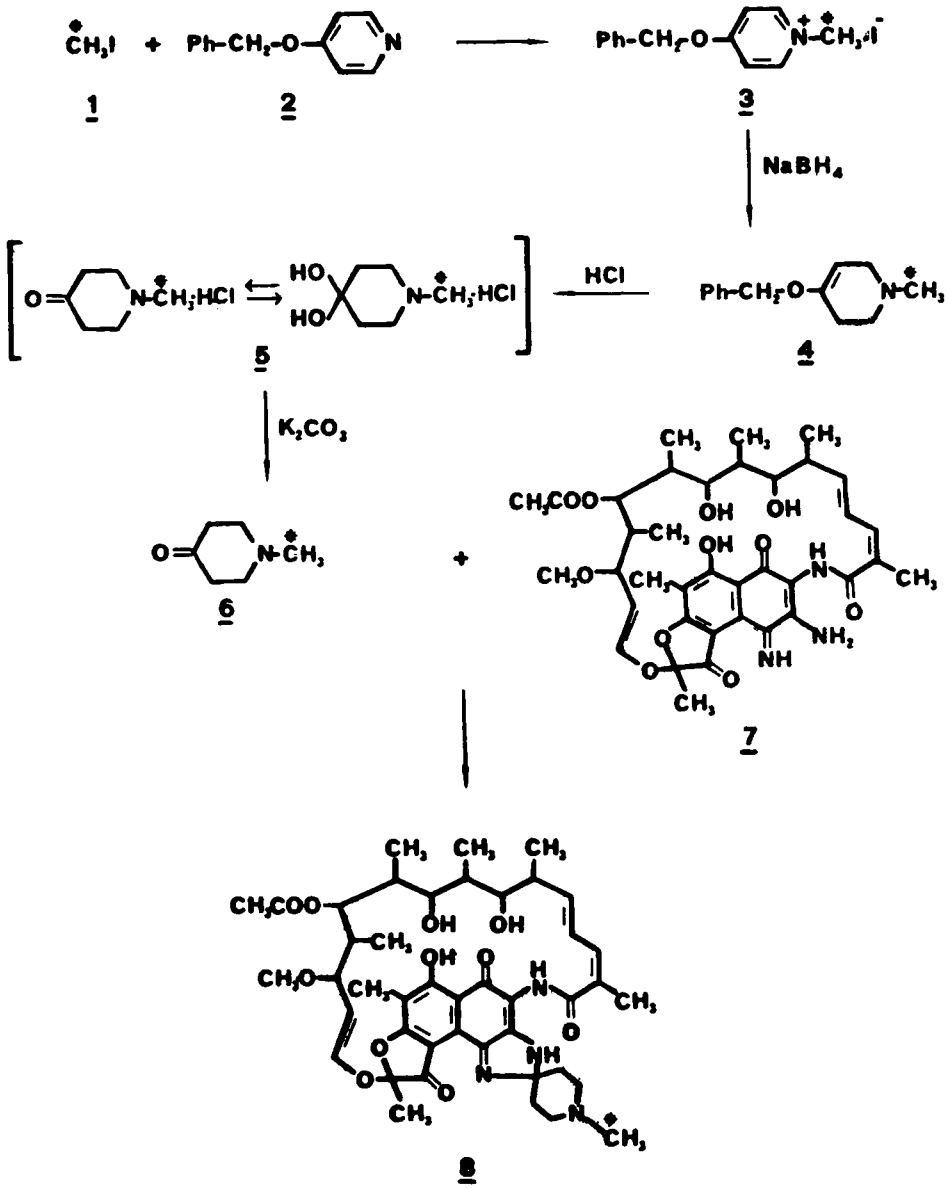
Tritiation by tritium gas exchange procedure (Wilzbach method) was unsuccessful for our purposes because the final molar specific activity was very low.

The easily available 3-amino-4-deoxo-4-imino-rifamycin S 7 and the "know how" (1) to obtain from this precursor the derivatives LM 118 and rifabutin (LM 427) suggested to consider such an intermediate as a possible approach for labelling the desired compounds with carbon-14.

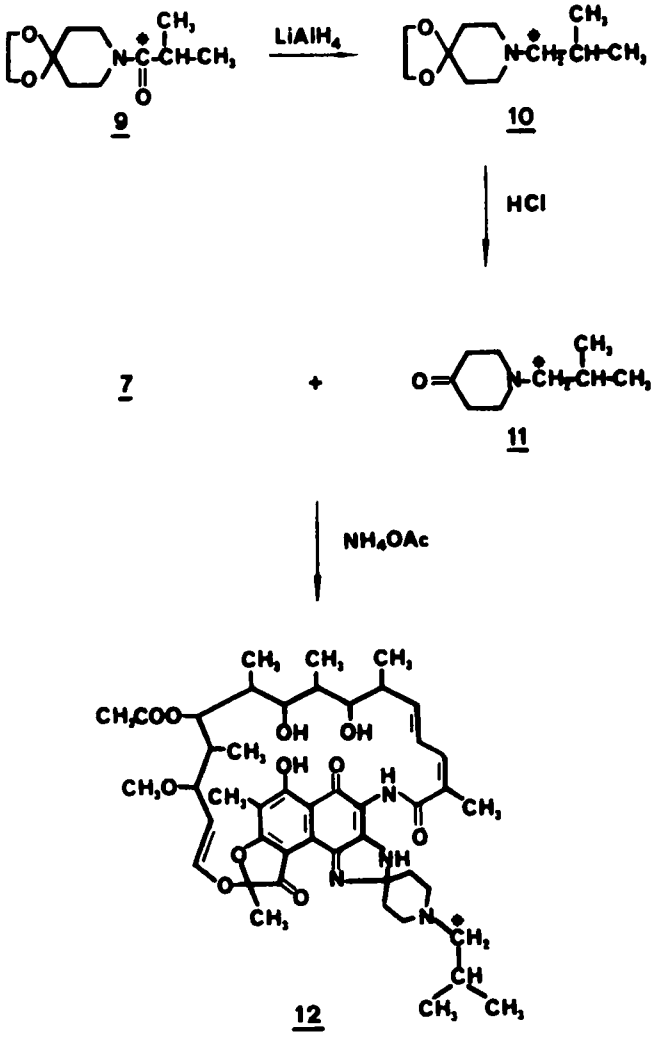
The recent identification of urinary metabolites of LM 427 in man (4) and "in vivo" experiments for both drugs (6) showed that the N-aliphatic side chains are sufficiently stable to metabolic attack.

Therefore synthetic procedures based on the introduction of the radiolabel in these side chains were chosen and the appropriate radioactive precursors for labelling LM 118 and LM 427 were investigated. The synthetic routes leading to the radioactive drugs are outlined in Schemes 1 and 2.

SCHEME 1



SCHEME 2



RESULTS AND DISCUSSION

All the synthetic steps necessary to obtain the labelled compounds were performed without purification of the intermediates because, with this procedure, it was possible to increase the radiochemical yields. A final chromatographic purification of the crude radioactive antibiotics, described in detail in the experimental part, was sufficient to obtain the pure compounds.

The precursor 6 for labelling LM 118 was prepared according to the procedure described by Lyle et al. (5), starting

from 4-benzyloxypyridine 2 via [^{14}C]methylpyridinium salt 3, which was converted into 1-[^{14}C]methyl-1,2,5,6,-tetrahydro-4-benzyloxy-pyridine 4 with $NaBH_4$. The further deprotection of the keto group with conc. HCl furnished the expected 1-[^{14}C]methylpiperidin-4-one 6(*). Crude 6, extracted with CH_2Cl_2 from an EXTRELUT Merck column furnished the expected [^{14}C]LM 118 8, 98% radiochemically pure, with a specific radioactivity of 440 MBq/mmol (11.9 mCi/mmol) in an overall radiochemical yield of 20% from [^{14}C]methyl iodide 1.

To obtain labelled rifabutin, the starting material,namely 1-(2 methyl[1-^{14}C]propanoyl)-piperazin-4-one ethylene ketal 9 was prepared by Amersham International plc according to our method by condensation of 8-aza-1,4-dioxaspiro-(4,5)-decane with [1-^{14}C]isobutyryl chloride. Compound 9 after reduction with $LiAlH_4$ and deprotection of the keto group, furnished compound 11, namely 1-(2-methyl-[1-^{14}C]propane)-piperazine-4-one.

(*) The small preparative scale of this compound (about 4 mmoles) made its isolation and purification by distillation difficult. On the other hand extraction of the crude from a water solution gave a product that was probably a hydrate that reacted very sluggishly with 3-amino-4-deoxy-4-imino-rifamycin S 7.

In this case, to avoid the possible formation of the hydrate, the crude 11 was directly trasferred, in a vacuum manifold, by distillation into the reaction flask containing the precursor 7. With this procedure [^{14}C]rifabutin, 97% radiochemically pure, with specific radioactivity 1.27 GBq/mmol (34.32 mCi/mmol) was obtained in an overall radiochemical yield of 37% from 9.

EXPERIMENTAL

Thin layer chromatography (TLC)

TLC was carried out using Merck silica gel F 254 200x50x0.25 mm plates. The eluting solvent systems were:

A) acetone: methanol	8:2	by volume
B) chloroform: methanol	8:2	by volume
C) chloroform: methanol	9:1	by volume
D) toluene: ethyl acetate : methanol	11:4:4	by volume

Electronic spectra were determined on a Perkin-Elmer 575 UV/VIS spectrophometer. Liquid scintillation counting was done with a Packard 300 C liquid scintillation counter using Rialuma (Lumac System A.G.) as liquid scintillation cocktail.

Radiochemical analysis of TLC plates was performed with a Berthold 2832 automatic TLC linear analyzer. High performance liquid chromatography (HPLC) was performed using a Perkin-Elmer 2x2 solvent delivery system with LC75 UV/VIS detector and Packard TRACE 7130 as radioactivity flow monitor.

Melting points were taken on a Kofler microscopic hot stage and are uncorrected.

[^{14}C]Methyl iodide and 1-(2-methyl[1-^{14}C]propanoyl)-piperazin-4-one ethylene ketal were purchased from Amersham International plc.

1-[^{14}C]Methyl-4-benzyloxypyridinium iodide (3)

[^{14}C]Methyl iodide 1 (12.2 mg, 0,086 mmoles, 185 MBq, [5 mCi]) diluted with "cold" 1 (30.2 mg, 0.213 mmoles) was added, with the high vacuum tecnique, to a solution of 4-benzyloxypyridine 2 (58.3 mg, 0.315 mmoles) in acetone (1 ml).

The reaction mixture, after stirring at room temperature for 12 hours, afforded a precipitate which was filtered, washed with cold acetone (~20 ml) and dried under vacuum yielding 182.4 MBq (4.93 mCi) of 3 (m.p. 140-141° C).

1-[^{14}C]Methyl-1,2,5,6-tetrahydro-4-benzyloxypiridine (4)

The compound 3 (182.4 MBq, [4.93 mCi]) was dissolved in absolute ethanol (1.5 ml) and sodium borohydride (119 mg, 3.13 mmoles) was added in small portions at room temperature.

At the end of the reaction, checked by radio-TLC (system A), the solution was concentrated to small volume and the residue was treated with 0.15 N K_2CO_3 (20 ml).

The resulting solution was absorbed onto an Extrelut R column and the crude compound 4 was extracted by eluting with chloroform (50 ml). Evaporation of the solvent furnished 4 which was used without further purification in the next step.

1-[^{14}C]Methylpiperidin-4-one (6)

Compound 4, prepared as described above, water (0.4 ml) and concentrated HCl (1 ml) were heated at reflux under stirring for 3 hours. The acid solution was then transferred into a separation funnel and extracted with chloroform (3x5 ml). The aqueous phase, after dilution with water (20 ml), was adjusted to pH 8 by 10% aqueous K_2CO_3.

The mixture was then absorbed onto an Extrelut R column. The column was eluted with dichloromethane (50 ml) and the solvent evaporated to give crude 6.

4-Deoxo-3,4-[2-spiro-(N-[^{14}C]methyl-4-piperidyl)]-(1H)-imidazo--(2,5-dihydro)-rifamycin S ([^{14}C] LM 118) (8)

3-Amino-4-deoxo-4-imino-rifamycin S 7 (314 mg, 0.44 mmoles) dissolved in dry THF (1 ml), was added under stirring at room temperature, to a concentrated methanolic solution of 6 and successively several small portions of 7 (up to 335 mg, 0.47 mmoles) were added over a period of five days. The reaction mixture was then kept at room temperature for 7 days. After completion of the reaction, as checked by radio-TLC (system B), the crude product was purified by column-chromatography on silica-gel (~35 g) eluting at first with chloroform (100 ml) and then with chloroform -methanol mixtures up to 4% of methanol (by volume). The fractions containing the expected compound [^{14}C] LM 118, were combined, concentrated and chromatographed on TLC plates (system B). The band corresponding to 8 was extracted from silica-gel with methanol. The recovered [^{14}C] LM 118 (36.5 MBq, [988 μCi]) had a specific activity of 440 MBq/mmol (11.9 mCi/mmol).
The UV absorbance in methanol at 276 nm ($E^{1\%}_{1cm}$ 393) was in agreement to that of an authentic sample. The radiochemical purity of [^{14}C] LM 118 was 98% (radio-TLC, system A:Rf 0.18 and system B:Rf 0.50). The overall radiochemical yield from 1 was 19.8%.

1-(2-Methyl[1-^{14}C]propane)-piperazin-4-one ethylene ketal (10)

An etheral solution (4 ml) of 130.6 mg (0.607 mmol), 1,295 GBq (35 mCi) of 9 and 90.3 mg (0.42 mmoles) of "cold" 9 was slowly added to a suspension of 112.7 mg (2.97 mmoles) of $LiAlH_4$ in ether (2 ml) and the mixture was stirred at 40°C for about 2 hours.

At the end of the reaction (checked by radio-TLC, system C), the mixture was cooled at 0°C and 4 ml of 10% H_2SO_4 were carefully added dropwise. After stirring 10 minutes at room temperature, 7 ml of 10% KOH were introduced into the flask. The resulting precipitate was filtered through a D3 sintered-glass filter and washed with small amounts of ether (5x5 ml). The biphasic filtrate was transferred into a 100 ml separating funnel and, after separation of the ethereal layer, the aqueous phase was extracted with CH_2Cl_2 (6x4 ml).

The combined organic extracts, were dried (anhydrous K_2CO_3) and concentrated to small volume to give 1.16 GBq (31.45 mCi) of crude 10 97% radiochemically pure (by radio-TLC, system C: Rf 0.37).

1-(2-methyl[1-^{14}C]propane)-piperazin-4-one (11)

Compound 10 (1.16 GBq, [31.45 mCi]), 2N HCl (2 ml) and acetone (~ 4 ml) were stirred and heated at reflux for about 14 hours. The cooled mixture was then adjusted to pH>9 with 10% aqueous KOH and extracted with CH_2Cl_2 (4x6ml). The combined organic fractions were dried (anhydrous K_2CO_3) and evaporated to small volume yielding 1.12 GBq (30.34 mCi) of compound 11, 75% radiochemically pure (by radio-TLC, system C: Rf 0.51) which was used without further purification in the next step.

4-Deoxo-3,4-[2-spiro-(N-2-methyl[1-^{14}C]propane-4-piperidyl)]-(1H)--imidazo-(2,5-dihydro)-rifamycin S ([^{14}C]rifabutin) (12)

Compound 11 (1.12 GBq [30.34 mCi]), obtained as described above was transferred, by the aid of the manifold, with the high vacuum technique, into a liquid nitrogen cooled 25 ml flask, containing 772.95 mg (1.089 mmoles) of 7, and NH_4OAC (13.05 mg) in anhydrous THF (4 ml).

The reaction mixture was kept at 5°C for 64 hours and then at room temperature, under stirring for about 7 hours. At the end of the reaction (checked by radio-TLC, system D), the solution was diluted with 20 ml of CH_2Cl_2.

The organic layer, washed with distilled water (15 ml) and dried over anhydrous Na_2SO_4, was purified by "flash-chromatography" on silica-gel Merck 60 (~30 g) employing a mixture of chloroform-acetone 8:2 (by volume) as eluting solvent. The fractions containing [^{14}C]rifabutin 12 were submitted to a further purification by preparative TLC using the system D as chromatographic eluent. The chromatographic band corresponding to 12 was removed and the product extracted from silica-gel with 200 ml of a mixture chloroform-methanol 4:1 (by volume). After evaporation of the solvent a UV spectrum showed that the residue still contained impurities. Therefore a further purification by"flash-chromatography" on silica-gel Merck 60 (~ 40 g) using two mixtures of chloroform:methanol in the ratios 9:1 and 8:2 (by volume), respectively as eluting solvent, was performed.

The fractions containing [^{14}C]rifabutin were combined yielding 493.9 MBq (13.35 mCi) of the expected product which migrates with an Rf identical to that for standard unlabelled compound when chromatographed besides or mixed with the unlabelled one. No radiolabelled or chemical (UV detection) impurities were detected in the [^{14}C]rifabutin sample. Radiochemical purity checked by radio - TLC (system D: Rf 0.54) and by radio-HPLC (retention time 10.8 min Merck RP 8 (10 μm); 4.6 mm ID x 24 cm; MeCN:0.02 M KH_2PO_4 buffer, pH 2.5; (1:1; by volume); flow rate 1 ml/min; UV detection 254 nm) was shown to be 97%. The radiochemical yield from 9 was ~ 38%.

ACNOWLEDGEMENTS

The authors thank Mrs. M.R. Tessera for helpful technical assistance.

REFERENCES

1. Marsili L., Pasqualucci C.R., Vigevani A., Gioia B., Schioppacassi G. and Orezzi G. - J. Antibiotics 34: 1033, (1981).

2. Sanfilippo A., Della Bruna C., Marsili L., Morvillo E., Pasqualucci C.R. and Ungheri D. - ibidem 33: 1193, (1980).

3. Della Bruna C., Schioppacassi G., Ungheri D., Jabes D., Morvillo E. and Sanfilippo A.- ibidem 36: 1502, (1983).

4. Vigevani A., Ballabio M., Gioia B., Marsili L., Vioglio S., and Franceschi G. - ibidem 38: 1799, (1985).

5. Lyle R., Adel R. and Lyle G. - J. Org. Chem. 24: 342, (1959).

6. Vioglio S., Gioia B., Vicario G.P., - unpublished data.